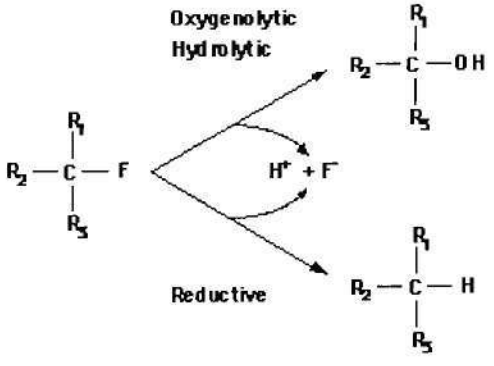
MICROBIAL TRANSFORMATION OF FLUORINATED ENVIRONMENTAL POLLUTANTS

Introduction

Fluorinated organic compounds (FOCs) have been extensively manufactured and used for the past several decades as surfactants, pesticide formulations, lubricants, refrigerants, fire retardants, drugs, cosmetics, paints, adhesives, etc. [1-7]. Recent studies indicated that FOCs are not biologically inert and exhibit toxic effects on humans and animals and impact overall ecosystem health. The strength of the carbon-fluorine bond and the anthropogenic origin of FOCs are often used to explain the inability of biological systems to degrade FOCs. However, there is convincing evidence that microbes are capable of degrading and detoxifying monofluorinated and polyfluorinated hydrocarbons, and that enzyme systems that break carbon-fluorine bonds exist [8-17]. Figure 1 summarizes the defluorination reactions that have been observed with microbial systems. The same mechanisms involved in breaking carbon-chlorine bonds exist to liberate fluorine from an organic molecule, suggesting that carbon-fluorine bonds are not inert, and biological attack is feasible.

Figure 1. Reactions carried out by bacterial cells, or parts thereof, that result in the liberation of fluoride from aromatic and aliphatic FOCs.



Oxygenolytic: Replacement of the fluorine substituent by a hydroxyl group derived from oxygen

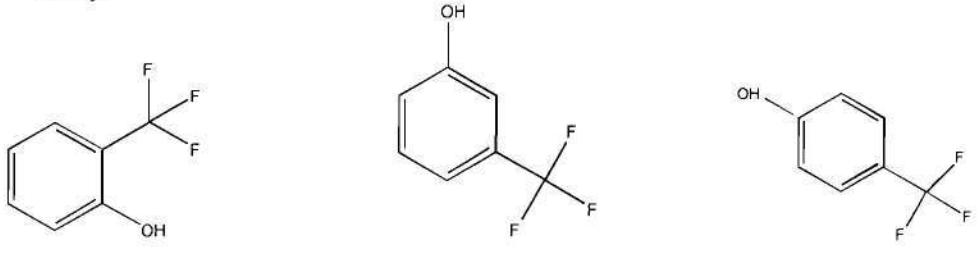
Hydrolytic: Replacement of the fluorine substituent by a hydroxyl group derived from water

Reductive: Replacement of the fluorine substituent by hydrogen

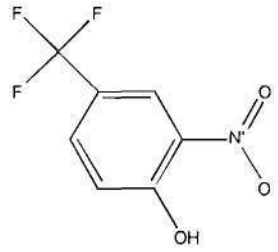
This research project explored the microbial degradation of alipahtic and aromatic hydrocarbons carrying one or more fluorine substituents. Among the FOCs investigated in this research project, monofluoroacetate (MFA) is a restricted use pesticide and has been placed in Toxicity Category I by EPA due to its high acute toxicity [18]. MFA has great physicochemical stability due to its fluorine-carbon bond, withstanding boiling and treatment with concentrated sulfuric acid [19]. The fate of MFA under anaerobic conditions has not been adequately explored.

4-nitro-3-trifluoromethylphenol (TFM) is the active ingredient in a restricted-use pesticide for controlling sea lamprey (*Petromyzon marinus*) in waters of the Great Lakes basin [20]. TFM has chemical characteristics that impart stability to organic compounds, and the fate of this compound and possible intermediates is poorly understood. TFM does not readily undergo chemical hydrolysis [21]. Despite its intensive use in the Great Lakes area, relatively few studies assessed the microbial degradation of TFM. Under anaerobic conditions, TFM can be reduced to 4-amino-3-trifluoromethylphenol (RTFM) over periods of 5 to 30 days by microbial activity [22-24]. RTFM was reported to be a stable intermediate, although there is some evidence suggesting that further breakdown might occur under anoxic conditions [20]. The enzymatic biological breakdown of TFM has been also investigated under aerobic conditions in natural water sediments [25]. No fluoride release was observed over a 10-week incubation period, and the trifluoromethyl-substituted aromatic ring system remained intact [20]. In this research, microbial defluorination of TFM and several other trifluoromethylphenol compounds, which share structural similarity with TFM (i.e., 2-, 3-, 4-trifluoromethylphenol and 2-nitro-4-trifluoromethylphenol; 2-TFMP, 3-TFMP, 4-TFMP and 2-N-4-TFMP, respectively), were explored (Figure 2).

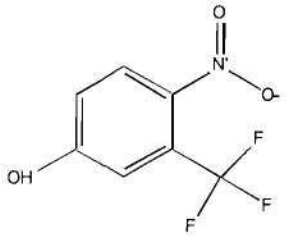
Figure 2. Structure of TFM and other trifluoromethylphenol compounds investigated in this study.



2-trifluoromethylphenol 3-trifluoromethylphenol 4-trifluoromethylphenol



2-nitro-4-trifluoromethylphenol



4-nitro-3-trifluoromethylphenol (TFM)

Another focus was on the microbial degradation of medium chain length fluorinated alkanes. Poly- and perfluorinated medium chain length fluorinated hydrocarbons are of particular concern due to their widespread distribution in the environment [4, 26]. To elucidate the microbial strategies that transform such aliphatic FOCs under aerobic conditions, 1-fluorodecane (1-FD) was chosen as a model compound, and the degradation of this compound was studied with *Pseudomonas* sp. strain 273. This organism is a gram-negative, aerobic bacterium that was isolated with 1,10-dichlorodecane (1,10-DCD) as the sole source of carbon and energy from a soil sample collected in Stuttgart, Germany. *Pseudomonas* sp. strain 273 released stoichiometric amounts of chloride from C_5 to C_{12} α,ω-dichloroalkanes. The best substrates for growth included C_9 to C_{12} terminal chloroalkanes, but this isolate also grew with nonhalogenated aliphatic compounds [27].

Objectives

This research effort explored the microbial degradation of fluorinated model compounds and environmentally relevant FOCs to improve our understanding of the fate of these chemicals in the environment. Specifically, the following objectives were addressed:

Objective 1: Explore if FOCs are reductively defluorinated and serve as metabolic terminal electron acceptors in anaerobic respiration.

Objective 2: Use model compounds to investigate the microbial strategies to degrade (poly)fluorinated hydrocarbons.

Objective 3: Demonstrate that environmentally relevant polyfluorinated and perfluorinated hydrocarbons can undergo microbially mediated defluorination/transformation reactions.

Approach and Analytical Procedures

a. Microcosms

Anaerobic microcosms were established with soil, sediment, and aquifer materials collected from 37 different locations (Table 1). Microcosms were established in 50-ml serum bottles, containing 5 g soil, sediment, or aquifer materials and 25 ml reduced basal salts medium under anoxic conditions [28]. The microcosms were amended with lactate as a source of reducing equivalents and carbon source and received hydrogen (3 ml) as additional electron donor. Environmentally relevant FOCs (trifluoroacetate, TFA; difluoroacetate, DFA; monofluoroacetate, MFA; 4-nitro-3-trifluoromethyl phenol, TFM) were added from anoxic, neutralized and sterilized stock solutions (100 mM) to final concentrations of 1 mM. Ethyl-4,4,4-trifluoroacetoacetate served as a model compound carrying a trifluoromethyl group, and was added at 1 mM concentration. Duplicates were prepared for each electron acceptor and autoclaved controls accompanied each experiment. The microcosms were incubated with the stopper down at 22°C in the dark without agitation.

Analytical procedures

Aqueous phase samples (1 ml) were withdrawn periodically and analyzed by ion chromatography (IC) to quantify TFA, DFA and MFA and to detect fluoride release. These compounds were analyzed with a Dionex DX-100 ion chromatograph (IC) with an IonPac® AS14A column (250 mm x 4 mm). The eluent was 100-fold diluted Dionex AS14A eluent concentrate (8 mM Na_2CO_3 + 1 mM $NaHCO_3$), which was pumped at 1.0 ml/min. Dilution series of each compound spanning a concentration range from 0.01 mM to 5 mM were prepared for generating the standard curves for IC analysis.

TFM and ethyl-4,4,4-trifluoroacetoacetate were analyzed by high performance liquid chromatograph (HPLC). TFM and ethyl-4,4,4-trifluoroacetoacetate were added as neat compounds. TFM was measured with a Waters 2996 Photodiode Array Detector and a Waters 717 Plus Autosampler (50 µl injection volume). The acidified (0.1% phosphoric acid, vol/vol) eluent (methanol/water, 60/40%; vol/vol) was pumped at a flow rate of 1.0 ml/min through a heated (30°C) Waters HPLC Nova-Pak C_{18} column (250 mm x 4.6 mm). Ethyl-4,4,4-trifluoroacetoacetate was measured with a Waters 2487 Dual λ Absorbance Detector and the same autosampler described above. The eluent was sulfuric acid (5 mM), which was

pumped at a flow rate of 0.5 ml/min through a heated (60°C) Bio-Rad Aminex HPX-87H column (300 mm x 7.8 mm). Dilution series of each compound were prepared for generating a standard curve spanning a concentration range from 0.01 mM to 2.0 mM. Before analysis, solid particles were removed from the samples by centrifugation in a microcentrifuge (14,000 rpm, 5 min at room temperature) followed by filtration (0.2 μm pore size).

Table 1. Sample materials tested for degradation/defluorination of FOCs

Site	Activity
Wastewater treatment facility, Atlanta GA	Defluorination of MFA and TFM
FMC Corp., CA (2 locations)	-[(a)]
Mangrove swamp, FL	-
Kalamazoo River, MI	-
Surgeon fish guts	TFM → RTFM
Young-Rainey Science, Technology, and Research (STAR) Center, FL (3 locations)	-
Stone Mountain State Park, GA	-
Milledgeville, GA (2 locations)	Defluorination of MFA and TFM
Visteon site, MN (2 locations)	-
Hydrite Chemical site, WI (2 locations)	-
Suzi Creek, South Korea	TFM → RTFM
Wastewater treatment facility, South Korea	TFM → RTFM
TRW Minerva, OH	Defluorination of TFM
Pinellas, FL (3 locations)	-
Patagonia, Chile (4 pristine locations)	Defluorination of TFM [(b)]
Buford Dam, GA (2 locations)	Defluorination of TFM
Occidental Chemical site, Montague, MI	-
Creek sediment, Urbana-Champaign, IL	Defluorination of MFA and TFM
Neckar River, Stuttgart, Germany	Defluorination of MFA and TFM
Savannah River, GA (2 locations)	Defluorination of TFM
Streambed sediment, Cancun, Mexico	Defluorination of MFA and TFM
Lake sediment, Fargo, ND	-

(a) - No activity
(b) Defluorination was observed in one out of four replicate microcosms.

b. Dechlorinating consortia

Five tetrachloroethene (PCE)-to-ethene dechlorinating consortia (BioDechlor INOCULUM, BDI, a commercially available bioaugmentation inoculum, and consortia CH, MB, GSI, and SHAW) were grown in 50-ml serum bottles containing 25 ml basal salts medium under anaerobic conditions [28]. The consortia were fed with lactate, except the BDI culture, which received hydrogen as additional electron donor. Gaseous chlorofluorohydrocarbons including 1,1-dichloro-2,2-difluoroethene, 1,2-dichloro-1,2-difluoroethene, 2-chloro-1,1-difluoroethene and chlorotrifluoroethene (CTFE) were added by syringe as a single dose of 0.5 ml per serum bottle. Trichlorofluoroethene (TCFE) was added to an aqueous concentration of 0.43 mM (2.5 μl per 25 ml of medium). TFA, DFA, MFA, TFM and ethyl-4,4,4-trifluoroacetoacetate were added at final concentrations of 1 mM. The degradation of 2-trifluoromethylphenol (2-TFMP), 3-trifluoromethylphenol (3-TFMP), 4-trifluoromethylphenol (4-TFMP) and 2-nitro-4-trifluoromethylphenol (2-N-4-TFMP) (1 mM each) was only evaluated with the BDI consortium. All cultures were initiated by adding 2% (vol/vol) of inoculum. In addition, FOC degradation was explored with the pentachloronitrobenzene dechlorinating PCNB consortium that has been maintained with methanol and glucose as electron donors for two years. Reduced, anaerobic basal salts medium was amended with 5 mM lactate, 5 mM butyrate, and 5 mM pyruvate and FOCs (i.e., TFA, DFA, MFA, TFM or ethyl-4,4,4-trifluoroacetoacetate, 1 mM each), and each vessel received a 2% (vol/vol) inoculum of the PCNB consortium.

Duplicates were prepared for each treatment and duplicate controls (i.e., cultures that received autoclaved inocula) accompanied each experiment. Culture bottles were incubated at 22°C in the dark. Samples (100 μl of headspace gas and 1 ml of liquid) were withdrawn periodically and analyzed using gas chromatography (GC) and IC, respectively.

Analytical procedures

1,1-dichloro-2,2-difluoroethene, 1,2-dichloro-1,2-difluoroethene, 2-chloro-1,1-difluoroethene, CTFE and TCFE were measured by using a HP6890 Series GC System Plus equipped with a HP-plot Q column (30 m x 530 mm x 40 mm film thickness) and a flame ionization detector (FID). Helium was used as the carrier gas. The column temperature was raised from

60°C to 200°C at a rate of 25°C/min with an initial hold of 6 min. Headspace samples were manually injected into a split injector operated at a split ratio of 50:1.

Microbial transformation of 1,1-dichloro-2,2-difluoroethene, 1,2-dichloro-1,2-difluoroethene, 2-chloro-1,1-difluoroethene, CTFE and TCFE was measured using a HP5890 Series II GC equipped with a J&W DB-5 column (30 m x 0.25 mm x 0.25 m film thickness) and a VG Instruments 70SE Mass Spectrometer.

Table 2. Dechlorinating consortia tested for defluorinating activity

Culture	Dechlorinating activity	Defluorinating activity
BDI	PCE → ethene	Defluorination of TFM
PCNB	PCNB → 2,5-dichloroaniline	Defluorination of TFM and MFA
MB	PCE → ethene	TFM → RTFM
CH	PCE → ethene	TFM → RTFM
GSI	PCE → ethene	TFM → RTFM
SHAW	PCE → ethene	None detected

c. Dechlorinating pure cultures

Four PCE-to-*cis*-1,2-dichloroethene dechlorinating pure cultures (*Desulfuromonas michiganensis* strain BB1, *Sulfurospirillum multivorans, Clostridium bifermentans* DPH-1, and *Geobacter* sp. strain SZ), a TCE-to-vinyl chloride dechlorinating pure culture (*Dehalococcoides* sp. strain FL2), one TCE-to-ethene dechlorinating pure culture (*Dehalococcoides* sp. strain GT) and one dichloroethene-to-ethene-dechlorinating pure culture (*Dehalococcoides* sp. strain BAV1) were grown in 160-ml serum bottles containing 100 ml reduced basal salts medium (Table 3). *Sulfurospirillum multivorans*, strain FL2 and strain BAV1 were provided with hydrogen (0.76 mM, aqueous concentration) as an electron donor and 2 mM acetate as a carbon source. Strain BB1 and *Geobacter* sp. strain SZ were fed with 2 mM acetate. Gaseous chlorofluorohydrocarbons (1,1-dichloro-2,2-difluoroethene, 1,2-dichloro-1,2-difluoroethene, 2-chloro-1,1-difluoroethene and CTFE) were added as a single dose of 0.5 ml per serum bottle by syringe. TCFE was added to a final aqueous concentration of 0.056 mM (1 µl per 100 ml of medium). TFA, DFA, MFA, TFM and ethyl-4,4,4-trifluoroacetoacetate were added at final concentrations of 1 mM. A 2-chlorophenol (2-CPh)

dechlorinating pure culture (*Anaeromyxobacter dehalogenans* strain 2CP-C) was tested with acetate (5 mM) as an electron donor and TFM (1 mM) as an electron acceptor. All culture vessels were seeded with a 2% (vol/vol) inoculum, and controls received 2% (vol/vol) of an autoclaved inoculum. Duplicates were prepared for each treatment. Culture vessels were incubated at 22°C in the dark. Samples (100 µl of headspace gas and 1 ml of liquid) were withdrawn periodically and analyzed using GC and IC, respectively.

Table 3. Pure cultures tested for defluorinating activity

Culture	Dechlorinating activity	Defluorinating activity
Desulfuromonas michiganensis strain BB1	PCE → *cis*-DCE	-
Sulfurospirillum multivorans	PCE → *cis*-DCE	-
Geobacter sp. strain SZ	PCE → *cis*-DCE	-
Dehalococcoides sp. strain GT	TCE → ethene	-
Dehalococcoides sp. stain BAV1	DCEs → ethene	-
Dehalococcoides sp. strain FL2	TCE → VC	-
Anaeromyxobacter dehalogenans strain 2CP-C	2-CPh → phenol	-
Clostridium bifermentans DPH-1	PCE → *cis*-DCE	-

- : no activity

d. Aerobic transformation of FOCs

Pseudomonas sp. strain 273, a 1,10-DCD degrading bacterium, was grown in 20-ml glass vials containing 2 ml basal salts medium with reduced chloride content under aerobic conditions [27]. The vials were closed with Teflon-lined rubber septa to prevent loss of volatile compounds. Various compounds including TFA, DFA, MFA, 4,4,4-trifluorobutyric acid, perfluoro-n-octanoate, ethyl-4,4,4-trifluoroacetoacetate, 1-fluorodecane (1-FD), decane, 1,10-DCD, and sebacic acid were provided as substrates. The degradation of MFA was also tested in cultures growing with of acetate or glucose. The vials received a 1% (vol/vol) inoculum from a strain 273 culture grown with 1-FD. 1-FD, decane, and 1,10-DCD formed a separate phase floating on top of the medium due to their low water solubility (i.e., light non-aqueous phase liquid, LNAPL). Due to the inhomogeneous distribution of these compounds in the culture medium, triplicate vessels were sacrificed and extracted with an organic solvent

(hexane) at each time point. Hexane (2 ml) was added to the culture vials for liquid-liquid extraction. Compounds in the hexane phase (i.e., 1-FD, decane and 1,10-DCD) were measured by GC using a liquid autosampler, and soluble compounds were analyzed by IC. All data points were averaged from triplicate cultures. Duplicate controls (no inoculum and autoclaved inoculum) accompanied each experiment. Culture vials were incubated at 35°C in the dark and shaken at 200 rpm.

Summary of Accomplishments

a. Anaerobic degradation of FOCs

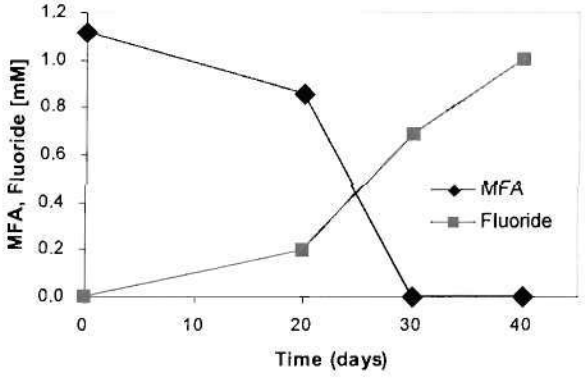
(i) Reductive defluorination of trifluoroacetate (TFA), difluoroacetate (DFA) and ethyl-4,4,4-trifluoroacetoacetate

Reductive defluorination of TFA, DFA, and ethyl-4,4,4-trifluoroacetoacetate was not observed in any of the microcosms established with sediment, soil, and aquifer materials listed in Table 1. Also, no fluoride release from these compounds occurred in cultures that were inoculated with the dechlorinating pure and mixed cultures.

(ii) Defluorination of monofluoroacetate (MFA)

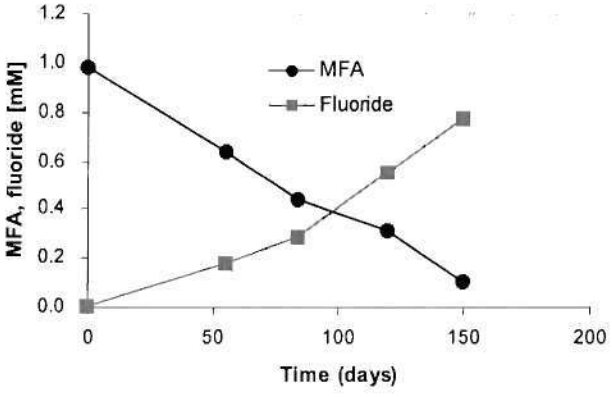
Anaerobic microcosms established with aquifer material collected from a chloroethene-contaminated aquifer in Milledgeville, GA, defluorinated MFA and stoichiometric amounts of fluoride were release. Subsequent transfers (3%, vol/vol) to fresh reduced basal salts medium amended with MFA and lactate yielded a sediment-free, MFA defluorinating culture (Figure 3). Defluorination of MFA occurred in four more anaerobic microcosms established with digestor sludge from a wastewater treatment facility in Atlanta, GA, creek sediment collected near the University of Illinois at Urbana-Champaign, creek sediment collected near Cancun, Mexico, and sediment from the Neckar River in Germany (Table 1).

Figure 3. Microbial defluorination of MFA in anaerobic 30-ml cultures following transfers from active microcosms established with sediment material from the Milledgeville site.



MFA degradation and fluoride release was also observed in the pentachloronitrobenzene dechlorinating PCNB consortium amended with butyrate as an electron donor, but the degradation rates were slow, requiring long incubation periods (>150 days) (Figure 4). No degradation of MFA was observed in all other microcosms, consortia and pure cultures tested.

Figure 4. Microbial defluorination of MFA by the PCNB consortium

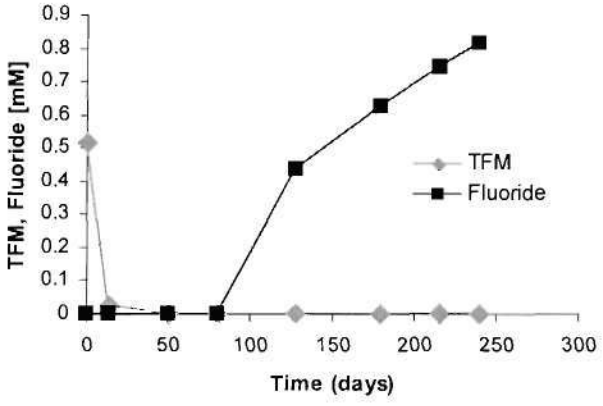


(iii) Degradation of 4-nitro-3-trifluoromethyl phenol (TFM)

TFM transformation was observed in microcosms established with twelve different sediments and aquifer materials (Table 1). These microcosms transformed TFM to RTFM over periods of 5 to 30 days. This reduction results in a loss of the characteristic yellow color

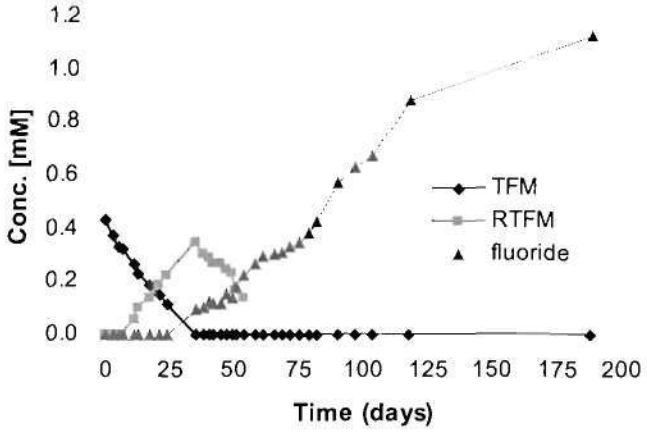
of TFM as measured by the decrease in absorption at 392 nm (Figure 5). Fluoride release from RTFM has been observed in microcosms derived from nine sampling sites (Table 1). Defluorination activity was maintained upon transfers to fresh medium amended with TFM and lactate, and sediment-free enrichment cultures were obtained. Figure 5 shows TFM transformation and fluoride release in sediment-free enrichment cultures derived from mud samples collected in Parque Nacional Torres Del Paine, Patagonia, Chile.

Figure 5. Microbial defluorination of TFM in microcosms established with samples from a pristine location in Patagonia, Chile



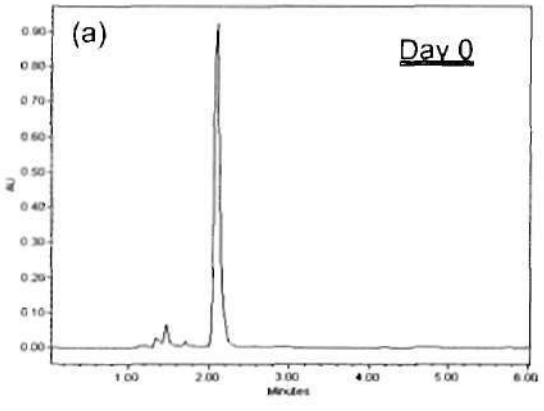
Six consortia (BDI, PCNB, MB, CH, GSI, and SHAW) reduced TFM to RTFM (Table 2). However, subsequent degradation of RTFM and the release of fluoride were observed only in the BDI and PCNB consortia. The BDI consortium has been sequentially transferred to fresh medium five times without loss of defluorinating activity (Figure 6). The concentration of RTFM is not shown in Figure 6 after 55 days of the incubation due to the overlap of the RTFM peak with an unknown peak in HPLC analysis. Fluoride release in the PCNB culture occurred slowly and required long incubation periods (>150 days).

Figure 6. Microbial defluorination of TFM by the BDI consortium following five consecutive transfers in basal salts medium amended with TFM

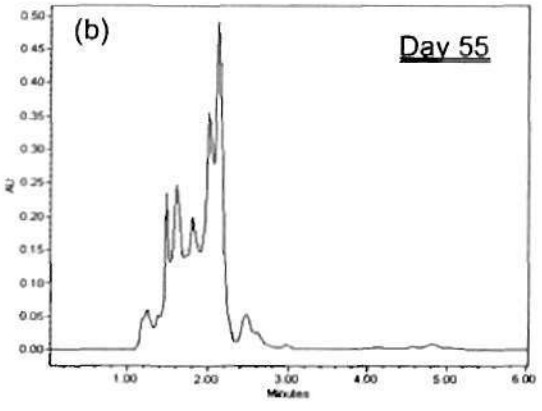


The BDI consortium also defluorinated 2-, 3-, 4-trifluoromethylphenol and 2-nitro-4-trifluoromethylphenol (2-TFMP, 3-TFMP, 4-TFMP and 2-N-4-TFMP, respectively). In 85 days following inoculation, 2.30 mM of fluoride was released from 1 mM of 2-TFMP, 0.05 mM from 3-TFMP, 0.83 mM from 4-TFMP, and 2.34 mM from 2-N-4-TFMP. For each trifluoromethylphenol compound, several novel peaks were observed in HPLC results, but none of the intermediates/end products have been identified (Figure 7).

Figure 7. HPLC chromatogram of transformation products formed during 4-TFMP defluorination by the consortium BDI (λ = 255 nm)



(a) 4-TFMP



(b) Products of 4-TFMP

b. Dehalogenation of chlorofluorohydrocarbons by chloroethene-dechlorinating consortia

*The chloroethene-dechlorinating consortia CH, MB, and GSI dechlorinated 1,1-dichloro-*2,2-difluoroethene to 2-chloro-1,1-difluoroethene and 1,1-difluoroethene (Table 4). No further transformation was observed, and no fluoride release occurred. The other two chloroethene-dechlorinating consortia (BDI and SHAW) dechlorinated 1,1-dichloro-2,2-difluoroethene to 2-chloro-1,1-difluoroethene and no further dehalogenation was observed over a 90 day incubation period.

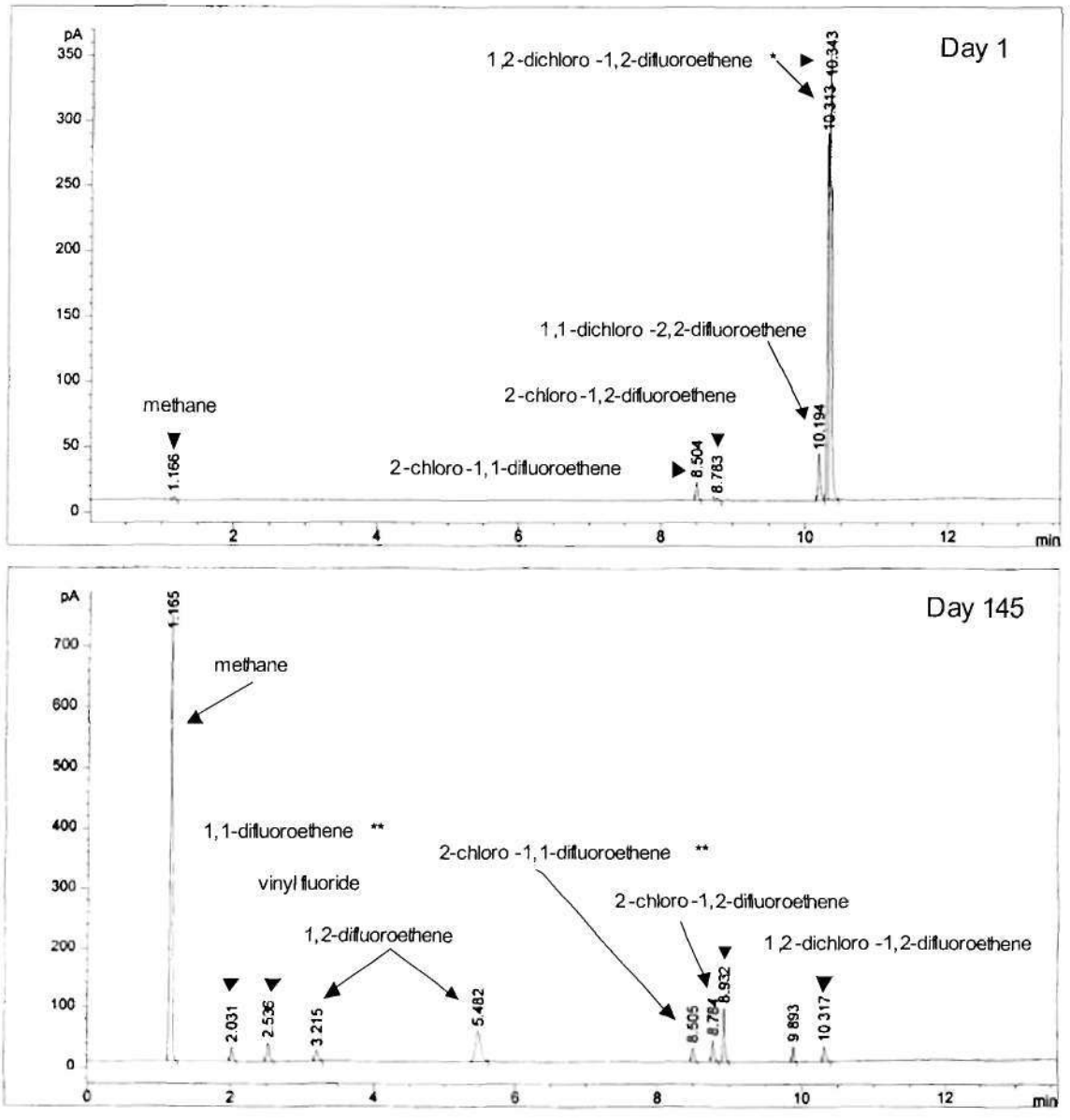
Reductive dehalogenation also occurred in cultures amended with *cis-/trans*-1,2-dichloro-1,2-difluoroethene (Figure 8). Consortia CH, MB and GSI dechlorinated *cis-/trans*-1,2-dichloro-1,2-difluoroethene to *cis-/trans*-2-chloro-1,2-difluoroethene, and *cis-/trans*-1,2-difluoroethene. Interestingly, vinyl fluoride was detected indicating that a reductive defluorination reaction occurred in these cultures. The formation of dehalogenation products was confirmed by GC/MS. Defluorination of vinyl fluoride to ethene or ethane was not observed. Although the GC system used for the analysis was able to separate *cis-/trans*-1,2-chloro-1,2-difluoroethene (Figure 8), quantification was not possible because standards were not available.

The effect of PCE on 1,2-dichloro-1,2-difluoroethene dehalogenation was explored with consortia CH, MB and GSI. All three consortia were grown with methanol as a source of reducing equivalents and PCE as an electron acceptor. After all PCE had been dechlorinated to ethene, transfers (3%, vol/vol) occurred to fresh medium amended with lactate (5 mM) and 1,2-dichloro-1,2-difluoroethene and/or PCE. In the presence of PCE, the lag time before the defluorination of 1,2-dichloro-1,2-difluoroethene occurred was reduced to 11 days in cultures of consortium CH compared with a 30-day lag in cultures that did not receive PCE. Consortium MB, however, responded differently to the presence of PCE, and 1,2-dichloro-1,2-difluoroethene defluorination was observed in 15 days in the absence of PCE but required 65 days in vessels that received PCE. In both cases, all PCE was consumed within 7 days and ethene was produced. The addition of PCE to consortium GSI had no effect on 1,2-dichloro-1,2-difluoroethene defluorination, which started after an 8-day lag period.

Both, the BDI and SHAW consortia dechlorinated *cis-/trans*-1,2-dichloro-1,2-difluoroethene only to *cis-/trans*-2-chloro-1,2-difluoroethene and no formation of vinyl

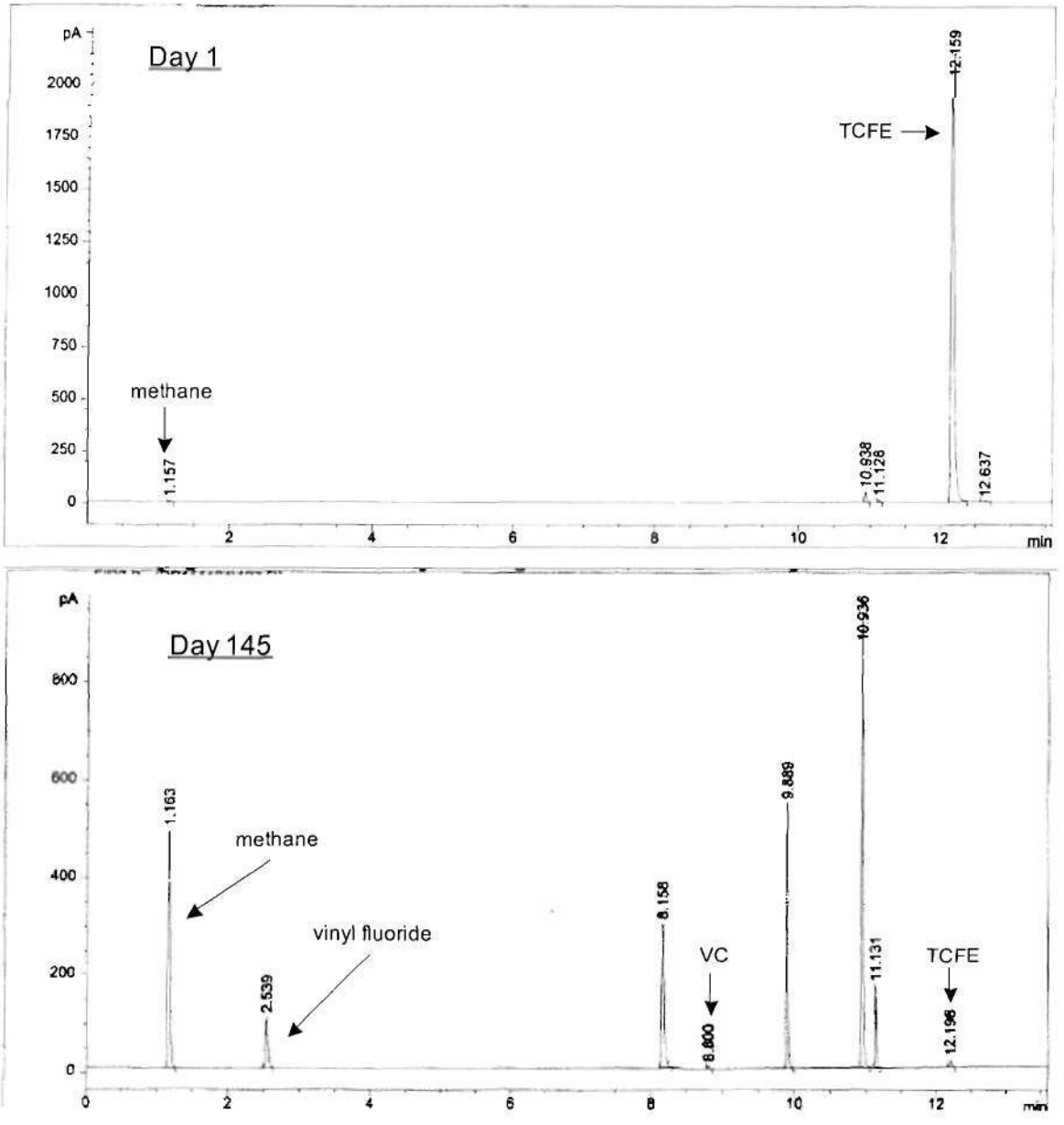
fluoride occurred during a 100-day incubation period. Consistent with the these observations was that the CH, MB and GSI consortia dechlorinated 2-chloro-1,1-difluoroethene to 1,1-difluoroethene, but the BDI and SHAW consortia failed to dehalogenate 2-chloro-1,1-difluoroethene. All five consortia (i.e., CH, MB, GSI, BDI and SHAW) dechlorinated CTFE to trifluoroethene. TCFE was dehalogenated by all cultures and a number of unidentified products were formed (Figure 9). Results of GC/MS analysis showed the formation of TCFE dechlorination products with one and two chlorines removed. Since each of these compounds have three isomers each, it was not possible to identify the exact structure of these intermediates/products.

Figure 8. Chromatogram of products formed during 1,2-dichloro-1,2-difluoroethene dehalogenation by the methanogenic consortium GSI



* 1,2-dichloro-1,2-difluoroethene (90 %) and 1,1-dichloro-2,2-difluoroethene (10 %).
** 2-chloro-1,1-difluoroethene and 1,1-difluoroethene are 1,1-dichloro-2,2-difluoroethene transformation products.

Figure 9. TCFE dehalogenation products formed by the methanogenic consortium GSI

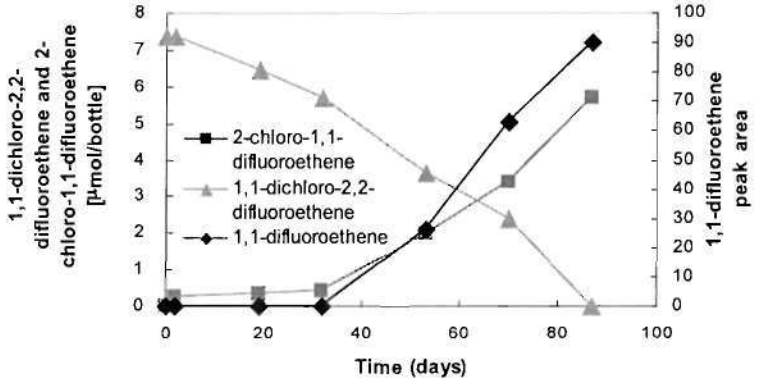


c. Dehalogenation of chlorofluorohydrocarbons by pure cultures that use chlorinated compounds as metabolic electron acceptors

Pure cultures capable of using chlorinated ethenes as metabolic electron acceptors (i.e., chlororespiring cultures) were challenged with chlorofluorohydrocarbons. Dehalogenation of

1,1-dichloro-2,2-difluoroethene was tested with three PCE-to-cis-DCE dechlorinating pure cultures (i.e., *Desulfuromonas michiganensis* strain BB1, *Sulfurospirillum multivorans*, and *Geobacter* sp. strain SZ) (Table 5). The experiments were performed in 160-ml serum bottles containing 100 ml of basal salts medium amended with 5 mM lactate as electron donor [28]. Cultures of *Desulfuromonas michiganensis* strain BB1 and *Geobacter* sp. strain SZ dechlorinated 1,1-dichloro-2,2-difluoroethene (30 μmol/bottle) to 2-chloro-1,1-difluoroethene (up to 10 μmol/bottle) in 4 days of incubation and 1,1-difluoroethene was produced (Table 5). All 1,1-dichloro-2,2-difluoroethene was converted to 2-chloro-1,1-difluoroethene, but not all 2-chloro-1,1-difluoroethene was converted to 1,1-difluoroethene for over 100 day incubation period. The dechlorination of 1,1-dichloro-2,2-difluoroethene by *Sulfurospirillum multivorans* is presented in Figure 10. No further transformation was observed, and no fluoride release occurred.

Figure 10. Dechlorination of 1,1-dichloro-2,2-difluoroethene by *Sulfurospirillum multivorans*



Reductive dechlorination also occurred in cultures of all three isolates amended with *cis*- and *trans*-1,2-dichloro-1,2-difluoroethene, but no fluoride release was observed even after extended incubation periods of 6 months. CTFE was dechlorinated by cultures of *Desulfuromonas michiganensis* strain BB1, *Sulfurospirillum multivorans* and *Geobacter* sp. strain SZ, and trifluoroethene accumulated. The formation of trifluoroethene was confirmed by GC-MS analysis. The highest CTFE dechlorination rates were measured in cultures of *Sulfurospirillum multivorans* whereas the transformation of CTFE in cultures of strain BB1 and strain SZ occurred at lower rates and was incomplete, even after prolonged incubation periods (Figure 11). TCFE was rapidly dechlorinated to several unidentified products by all

three cultures (Table 5 and Figure 12), but no vinyl fluoride was formed. No dehalogenation was observed in cultures of *Dehalococcoides* sp. strain FL2, *Dehalococcoides* sp. strain BAV1 and *Dehalococcoides* sp. strain GT suggesting that these populations cannot transform these chlorofluorohydrocarbons.

Figure 11. Dechlorination of CTFE by *Desulfuromonas michiganensis* strain BB1, *Sulfurospirillum multivorans* and *Geobacter* sp. strain SZ

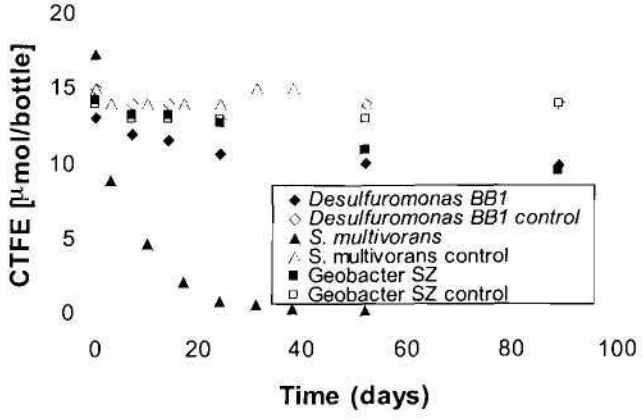
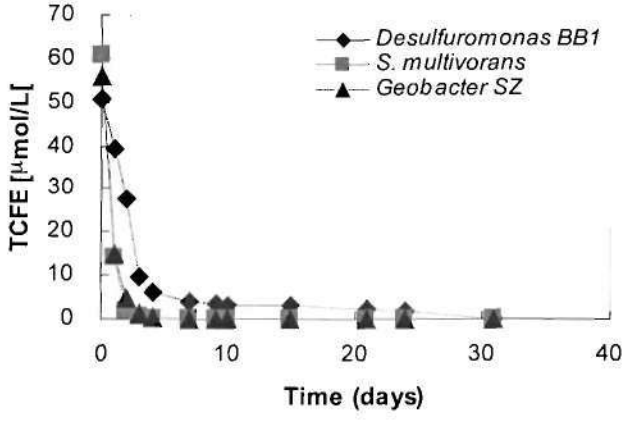


Figure 12. Dechlorination of TCFE by *Desulfuromonas michiganensis* strain BB1, *Sulfurospirillum multivorans* and *Geobacter* sp. strain SZ



Clostridium bifermentans DPH-1 dechlorinated 1,1-dichloro-2,2-difluoroethene only to 2-chloro-1,1-difluoroethene and *cis-/trans*-1,2-dichloro-1,2-difluoroethene to *cis-/trans*-2-chloro-1,2-difluoroethene. *C. bifermentans* DPH-1 did not dehalogenate 2-chloro-1,1-difluoroethene and CTFE. TCFE was dechlorinated but no vinyl fluoride was formed and two unidentified products were observed by GC analysis.

Table 4. Products of chlorofluorohydrocarbon dehalogenation by chloroethene-dechlorinating consortia

Compound / Consortium	1,1-dichloro-2,2-difluoroethene	*cis-/trans*-1,2-dichloro-1,2-difluoroethene	2-chloro-1,1-difluoroethene	chlorotrifluoroethene	trichloro-fluoroethene
BDI	2-chloro-1,1-difluoroethene	*cis-/trans*-1-chloro-1,2-difluoroethene	-	trifluoroethene	5 peaks (not identified)
CH	2-chloro-1,1-difluoroethene 1,1-difluoroethene	*cis-/trans*-1,2-difluoroethene vinyl fluoride	1,1-difluoroethene	trifluoroethene	vinyl fluoride
MB	2-chloro-1,1-difluoroethene 1,1-difluoroethene	*cis-/trans*-1,2-difluoroethene vinyl fluoride	1,1-difluoroethene	trifluoroethene	vinyl fluoride
GSI	2-chloro-1,1-difluoroethene 1,1-difluoroethene	*cis-/trans*-1,2-difluoroethene vinyl fluoride	1,1-difluoroethene	trifluoroethene	vinyl fluoride (5 peaks not identified)
SHAW	2-chloro-1,1-difluoroethene	*cis-/trans*-1-chloro-1,2-difluoroethene	-	trifluoroethene	-

- No dehalogenation

Table 5. Products of chlorofluorohydrocarbon dehalogenation by pure cultures

Compound / Isolate	1,1-dichloro-2,2-difluoroethene	*cis-/trans*-1,2-dichloro-1,2-difluoroethene	2-chloro-1,1-difluoroethene	chlorotrifluoroethene	trichloro-fluoroethene
Desulfuromonas BB1	2-chloro-1,1-difluoroethene 1,1-difluoroethene	*cis-/trans*-2-chloro-1,2-difluoroethene	1,1-difluoroethene	trifluoroethene	4 peaks (not identified)
S. multivorans	2-chloro-1,1-difluoroethene 1,1-difluoroethene	*cis-/trans*-2-chloro-1,2-difluoroethene	1,1-difluoroethene	trifluoroethene	5 peaks (not identified)
Geobacter SZ	2-chloro-1,1-difluoroethene 1,1-difluoroethene	*cis-/trans*-2-chloro-1,2-difluoroethene	1,1-difluoroethene	trifluoroethene	4 peaks (not identified)
C.bifermentans	2-chloro-1,1-difluoroethene	*cis-/trans*-2-chloro-1,2-difluoroethene	-	-	2 peaks (not identified)
Dehalococcoides GT	-	-	-	-	-
Dehalococcoides BAV1	-	-	-	-	-
Dehalococcoides FL2	-	-	-	-	-

- No dehalogenation

d. Aerobic degradation of fluorinated alkanes

Strain 273 grew with 1-FD as the sole source of carbon and energy, and stoichiometric amounts of fluoride were released into the growth medium (Figure 13) [29]. No intermediates were detected during growth with 1-FD. Strain 273 did not grow with MFA as the sole source of carbon and energy. However, when the organism was grown with a mixture of 1-FD and MFA, growth occurred and both 1-FD and MFA were degraded (Figure 14).

Figure 13. Degradation and defluorination of 1-FD by *Pseudomonas* sp. strain 273

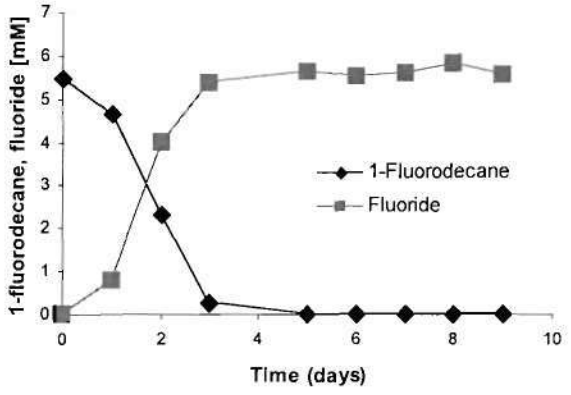
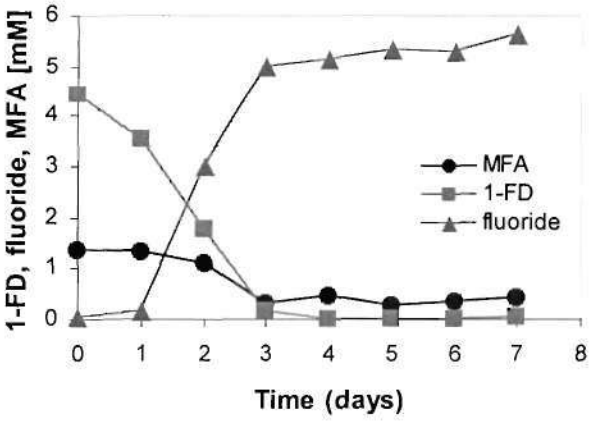


Figure 14. Degradation of 1-FD and MFA by *Pseudomonas* sp. strain 273



Strain 273 also grew readily with decane, sebacic acid, glucose, and acetate as sole sources of carbon and energy. When MFA was provided together with each of these substrates, growth occurred at the expense of decane (Figure 15), sebacic acid, and glucose, and MFA was degraded

concomitantly and fluoride was released. Similar results were obtained in acetate-fed cultures, although repeated additions of acetate (5 mM) were required to achieve stoichiometric fluoride release from MFA (Figure 16). The headspace of each culture vessel was purged with sterile air for 2-3 minutes following each acetate addition to ensure aerobic conditions. As shown in Figure 16, no MFA was detectable after 10 days, and approximately 0.65 mM fluoride was released from 1 mM of MFA after the 4-week incubation period. No other (fluorinated) intermediates were detected by IC and LC/MS analyses.

Figure 15. Degradation of decane and MFA by *Pseudomonas* sp. strain 273

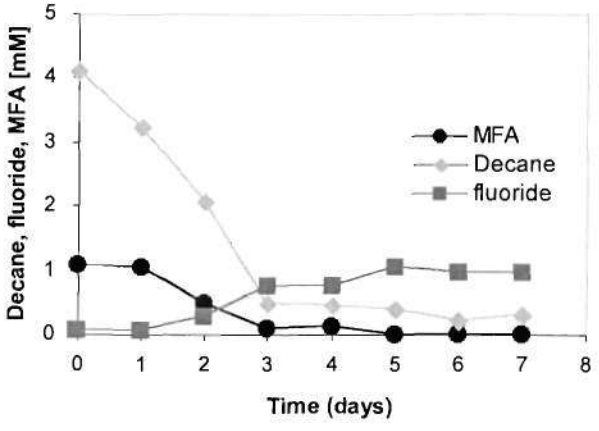
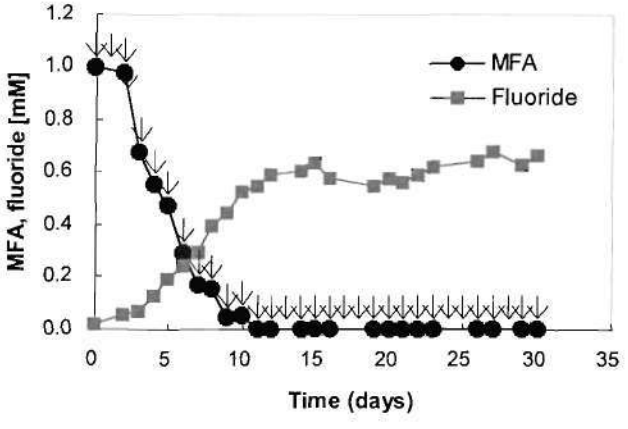


Figure 16. Degradation of acetate and MFA by *Pseudomonas* sp. strain 273.



↓: addition of 5 mM acetate

When the MFA concentration was increased to 2 mM, strain 273 grew on the expense of 1-FD (Figure 17) and decane (Figure 18), but no MFA consumption occurred and no fluoride was released.

Figure 17 Degradation of 1-FD in the presence of 2 mM MFAin cultures of *Pseudomonas* sp. strain 273

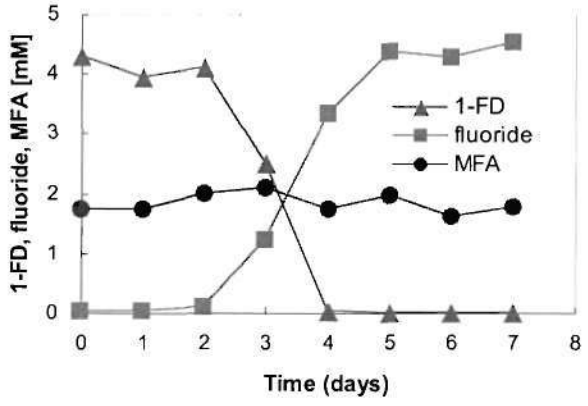
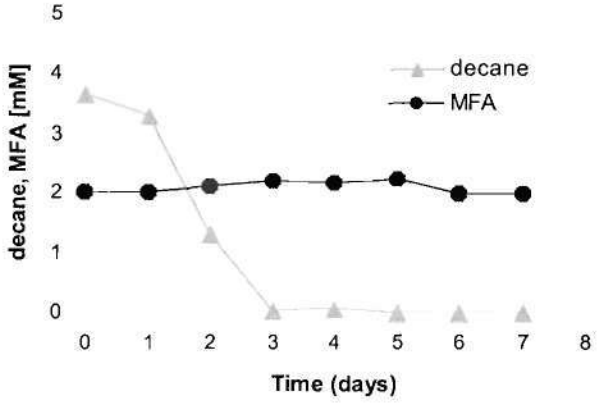


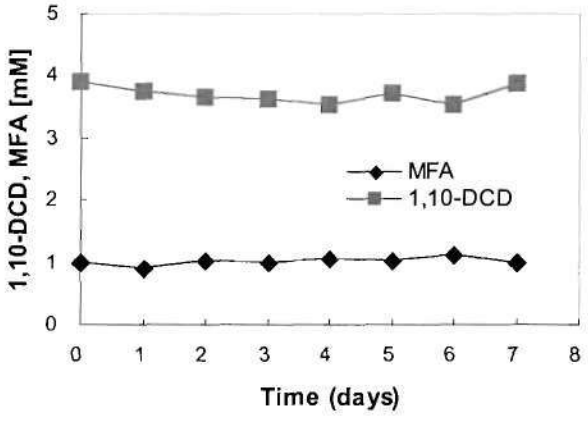
Figure 18. Degradation of decane in the presence of 2 mM MFA in cultures of *Pseudomonas* sp. strain 273



Strain 273 grew with 1,10-dichlorodecane (1,10-DCD) as a sole source of carbon and energy and released stoichiometric amounts of chloride [27]. When the organism was grown with a mixture of 1,10-DCD and MFA, no growth was observed and neither 1,10-DCD nor MFA

were degraded (Figure 19) [30]. While MFA had no inhibitory effect on growth of strain 273 with 1-FD, decane, sebacic acid, glucose and acetate, MFA completely inhibited growth with 1,10-DCD. Strain 273 metabolizes (halogenated) alkanes via β-oxidation, indicating that MFA or MFA-CoA are possible intermediates in the degradation of 1-FD. It is interesting to note MFA inhibited growth with 1,10-DCD but not with 1-FD or other substrates tested. This observation is relevant, and emphasizes that the complex interaction between microbes and their substrates must be understood before meaningful and reliable predictions on the fate of FOCs in the natural environment are possible.

Figure 19. *Pseudomonas* sp. strain 273 failed to degrade 1,10-DCD in the presence of 1 mM MFA



Strain 273 failed to grow with MFA, DFA and TFA as sole sources of carbon and energy. When the organism was grown with a mixture of 1-FD plus DFA or TFA, growth occurred at the expense of 1-FD but DFA or TFA were not transformed. Similarly, neither DFA nor TFA degradation occurred in cultures growing with decane, 1,10-DCD, sebacic acid, glucose or acetate.

No growth occurred in cultures of strain 273 amended with perfluoro-n-octanoate, 4,4,4-trifluorobutyric acid, or ethyl-4,4,4-trifluoroacetoacetate as the sole sources of carbon and energy. In the presence of 1-FD, growth occurred on the expense of 1-FD, and perfluoro-n-octanoate (1 mM), 4,4,4-trifluorobutyric acid (1 mM) and ethyl-4,4,4-trifluoroacetoacetate (1 mM) were partially degraded and additional fluoride (0.3 mM) was released. Similar results were obtained

with 1,10-DCD as the primary substrate. When the organism was grown with decane in the presence of perfluoro-n-octanoate (1 mM), 4,4,4-trifluorobutyric acid (1 mM) and ethyl-4,4,4-trifluoroacetoacetate (1 mM), decane was consumed but the FOCs were not degraded and no fluoride release occurred.

Publications/Presentations:

Results from this research were presented at the Annual Meeting of the American Society for Microbiology in New Orleans in May 2004, at the National Meeting of the American Chemical Society in Philadelphia in August 2004, and at the Annual Meeting of the American Society for Microbiology in Atlanta in June 2005.

1. Song, R., Sohn, R, and F.E. Loeffler. 6/2005. Anaerobic microbial transformation of monofluoroacetate and 4-nitro-3-trifluoromethyl phenol, abstract Q077. *In* Abstracts of the 105th General Meeting of the American Society for Microbiology, Atlanta, GA.
2. Song, R., and F. E. Loeffler. 8/2004. Degradation of 1-fluorodecane and monofluoroacetate by *Pseudomonas* sp. strain 273, abstract ENVR 221. *In* Abstracts of the 228th American Chemical Society National Meeting, Philadelphia, PA.
3. Song, R., and F. E. Loeffler. 5/2004. Aerobic degradation of fluorinated alkanes by *Pseudomonas* sp. strain 273, abstract Q456. *In* Abstracts of the 104th General Meeting of the American Society for Microbiology, New Orleans, LA.

Manuscripts in preparation:

1. Song, R., and F. E. Loeffler. 2005. Aerobic degradation of 1-fluorodecane and cometabolic defluorination of MFA by *Pseudomonas* sp. Strain 273. In preparation.
2. Song, R., and F. E. Loeffler. 2005. Dehalogenation of chlorofluorohydrocarbons by chloroethene-respiring consortia and pure cultures. In preparation.
3. Song, R., Sohn, R, and F. E. Loeffler. 2005. Anaerobic microbial degradation of polyfluorinated hydrocarbons. In preparation.

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Fluorinated hydrocarbons, sediments, human and ecosystem health, biodegradation, detoxification, restoration, halorespiration, defluorination.

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30. Song, R., and F. E. Loeffler. *Aerobic degradation of fluorinated alkanes by Pseudomonas sp. strain 273*, abstract Q456. 2004, 104th General Meeting of the American Society for Microbiology, New Orleans, LA.